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Eliciting Action Potentials from Epidermal Stimulation of Skin Receptors Using Ultrashort Laser Pulses

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14. ABSTRACT

Measurements of laser stimulated action potentials in the sciatic nerve of leopard frogs (*Rana pipiens*) were made using two ultrashort pulsed infrared lasers. The dorsal sides of the frog's hind limbs were exposed to 1540 nm and 1064 nm wavelengths at three separate spot sizes: 2 mm, 3 mm, and 4 mm. Energy density thresholds were determined for eliciting an action potential at each experimental condition. Results from these exposures showed similar evoked potential thresholds for both wavelengths. Skin ablation was observed at temperature increases as low as 0.7 °C, so we believe the primary skin damage mechanism to be stress confinement. Determining the method of receptor activation was outside the scope of this study. While the exact mechanism still remains unknown, it is possible to elicit action potentials from transdermal exposures of ultrashort lasers.

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Abstract

Measurements of laser stimulated action potentials in the sciatic nerve of leopard frogs (*Rana pipiens*) were made using two ultrashort pulsed infrared lasers. The dorsal sides of the frog's hind limbs were exposed to 1540 nm and 1064 nm wavelengths at three separate spot sizes: 2 mm, 3 mm, and 4 mm. Energy density thresholds were determined for eliciting an action potential at each experimental condition. Results from these exposures showed similar evoked potential thresholds for both wavelengths. Skin ablation was observed at temperature increases as low as 0.7 °C, so we believe the primary skin damage mechanism to be stress confinement. Determining the method of receptor activation was outside the scope of this study. While the exact mechanism still remains unknown, it is possible to elicit action potentials from transdermal exposures of ultrashort lasers.

Background

Electrical stimulation is commonly used to stimulate action potentials in neurons for both medical and research applications. Electrical signals are applied to a nerve, initiating the voltage change that will start a chain reaction along the axon. Once began, the signal is passed along the axonal tract. Unfortunately, electrical stimulation systems possess characteristics that create problems in this kind of work. Besides low spatial specificity (electrical stimulation will activate several nerve tracts simultaneously), difficulties include tissue damage from electrode installation and unnatural action potential responses (Izzo, et al., 2006). Recent studies have found that a laser source can be used to induce an action potential in the nervous system as well as, if not better than, electrical methods. Kao et al. have shown that there are few differences between optical and electrical stimulation on the activation of the nerves (Kao, Wells, & Jansen, 2005). Laser excitation of neural activity provides a contact-free, spatially selective, artifact-free method of stimulation without incurring tissue damage (Kao, Wells, & Jansen, 2005). The small spot sizes used by laser systems allow for pin point accuracy when stimulating nerve tracts and the low irradiance levels help to minimize introduction of extra energy into the action potential response. The amplitude of an action potential is directly proportional to the strength of its triggering event so the lower energies used by laser stimulations are very important in achieving greater specificity. Most of the studies were performed directly on the nerves and were within laser-tissue interaction parameters that led Wells to suspect thermal confinement mechanisms for action potential elicitation.

Clinically, indirect stimulation of nerves has been conducted by radiating the skin with lasers (typically a CO_2 or visible wavelength laser) and activating skin nerve fibers. This technique is typically used for determining pain thresholds, pain desensitization studies, and physiological studies of nociceptive pathways. (Perchet et al., 2008; Kneebone, 2007; Moore, 2004; Arendt-Nielsen, 1988) To date, we have been unable to find any data on laser-evoked potentials using ultrashort lasers.

This work is a pilot study to determine if action potentials can be induced using near-infrared ultrashort pulses directed through the skin of an animal. Much work has been performed by this laboratory into the damage thresholds of ultrashort lasers, and this has led to a desire to clarify whether or not these lasers are causing any unseen secondary reactions at low energy levels. Unlike longer pulsed lasers which operate in thermal confinement parameters, ultrashort pulses

operate within stress confinement parameters. Thus, any reaction could be thermal, mechanical, or a combination of these or other mechanisms. If they can indeed elicit action potentials, further work will be required to determine the exact method.

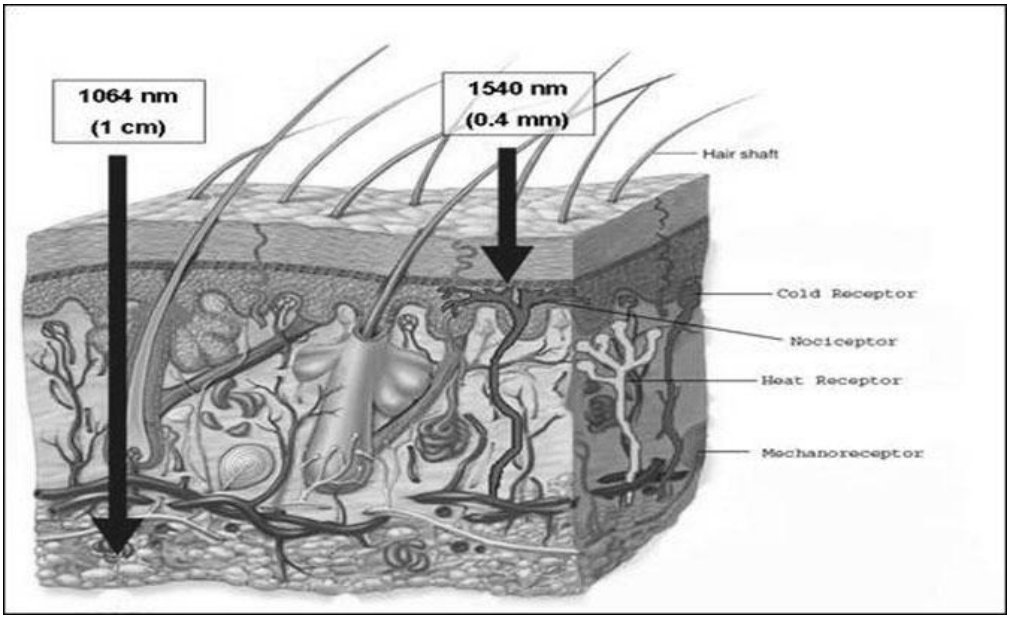

Figure 1: Illustration of laser energy penetration and nerve receptor locations in skin layers.

Objectives

The goal of this work was to determine the feasibility of an electro-potential response of neural receptors due to ultrashort pulsed laser exposures.[†] This was tested during an experiment performed on Leopard Frogs. An electrode was placed in the sciatic nerve of the frogs to record action potentials elicited from laser exposures on the surface skin of the calf. Three spot sizes and two different infrared wavelengths were used in the study. The data was then analyzed to determine threshold values for action potential stimulations.

Methods

Leopard Frogs

In vivo sciatic nerve experiments were performed using Leopard Frogs (*Rana pipiens*) from the Carolina Biological Supply Company in North Carolina. The frogs ranged in torso length from 3 to 4 inches and were euthanized via a double pithing technique. To maintain a constant body temperature during the experiment, the cold-blooded frogs were placed on a saline bag that had

[†] * The animals involved in this study were procured, maintained, and used in accordance with the Federal Animal Welfare Act, "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources National Research Council, and DoD Regulation 40-33 Secnavinst 3900.38C AFMAN 40-401(1) DARPAINST 18 USUHSINST 3203 "The Care and Use of Laboratory animals in DOD Programs." Brooks City-Base, TX has been fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1967. (Protocol HEDO-06-12).

been warmed to approximately 20-22 ˚C. This was to ensure that the nerve receptors remained within their effective ranges and that the frogs were not negatively affected by cold ambient temperatures in the lab.

Since the subject was an amphibian, the water content of the skin was very high. In order to minimize variability in the data obtained during this experiment, saline was periodically applied to the skin to maintain hydration. Excess solution was blotted off using gauze.

Nerve Preparation

Nerve preparation started by making a centrally located incision from the knee to the upper thigh, removing the skin from the dorsal side and exposing the trunk of the nerve at the knee. Muscular fascia was incised and removed to expose the rest of the nerve. A piece of latex was placed between the nerve and the underlying muscle in order to minimize any collateral electrical signals. After the nerve was prepared, insulated stainless steel needle electrodes (Chalgreen Enterprises, Inc. 111-637-24TP, Disposable, Monopolar EMG Needle Electrodes, 37mm x 26 gauge) were inserted into the nerve located approximately 15 mm above the knee. Baseline data was collected to verify the system's isolation from other electrical sources and to ensure correct electrode placement. To initialize each experiment, the sciatic nerve was directly stimulated by the laser to verify that the electrode was reading correctly and that the nerve had not been damaged by the insertion. Compound nerve action potential (CNAP) responses were recorded with BioPac Systems Inc. MP100 interfaced to a computer running Acknowledge software v3.73. The CNAP is the algebraic sum of many individual "all-or-none" action potentials arising more or less simultaneously in a large number of individual axons (Physiology Dept., McGill University, 2005). All action potentials are measured using a differential medical amplifier and extracellular recording electrodes, which measure the summed electrical response of all excited axons in the nerve. The recordings for this project were manually triggered prior to the exposure and recorded 5 seconds of data. All signals were amplified 1000 times and electrically filtered with a 50 to 5000 Hz bandpass filter.

Once the initial direct testing of the sciatic nerve had been conducted, the laser was focused on the animal's calf. The surface of the skin was randomly irradiated using varying energy levels, with one second in between each shot. This lag time was necessary to prevent overheating of the Er:Glass laser and was maintained for both lasers to eliminate unnecessary variation between the exposure procedures.

Laser Set-Up

Optical stimulation was performed using two infrared laser sources. A Q-switched Nd:YAG laser emitting 1064 nm was first used to verify experimental methods. The Nd:YAG laser was pulsed at a repetition rate of 10 Hz with a pulse duration of 15 ns. The energy of the laser was controlled using a half wave plate and a polarizing beam splitter, and the generated pulses were sampled using a 90/10 non-polarizing beamsplitter by an Ophir Laser Star energy meter using #1Z0230 power head. The laser was then directed into a faraday cage where it would be focused into the desired spot size using a 500 mm bi-convex lens. (Figure 2)

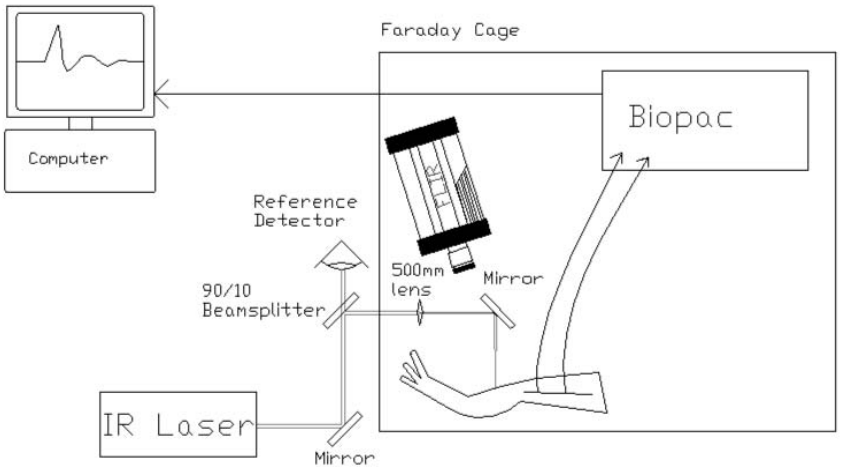

Figure 2: Generic schematic of experimental set-up.

Each infrared laser used possessed a Gaussian spatial beam profile. Beam diameters and profiles were measured using linagraph laser burn paper. Using only one pulse per exposure, three beam diameters were used during the experiment: 2 mm, 3 mm and 4 mm.

Once the methods were verified with the Nd:YAG laser, an Er:Glass (Erbium-glass) laser emitting 1540-nm was employed to further evaluate optical stimulation and for statistical comparison with another wavelength. The Er:Glass laser was mechanically q-switched to a pulse duration of 55 ns. Due to the system's high energy, only 1 shot per minute is allowed. The energy of the laser is varied by adjusting the flash lamp energy. Again the beam was directed into the faraday cage and focused to the same spot sizes as with the 1064-nm Nd:YAG laser: 2 mm, 3 mm and 4 mm.

Data showed considerable variability between the animals in respect to the pigmentation placement. While melanin has only a small role in energy absorption at infrared wavelengths, early skin exposures showed noticeable differences between skin damage threshold energy levels for dark and light skin patches. Thus, for greater consistency, only lightly pigmented skin data was used in the analysis. Each AP_{50} is represented in units of fluence (J/cm^2).

Probit Analysis

Probit analysis was developed in order to analyze discrete data collected by experiments involving threshold response rate in biological systems. This is computed using the EZ-Probit program designed by Dr. Clarence Cain and Capt. Lonnie Manning at Brooks City- Base in San Antonio, Texas (Cain & Manning, 1996). This method has been employed as a statistical tool to determine the probability of dose-response curves for action potential (AP) responses in the sciatic nerve. In this case the thresholds probabilities are reported as AP_{50} -- the radiant exposure dose which has a 50% probability of creating a response. The values presented here are for 100% probability, without consideration of additional experimental uncertainties. Also, the slope of the probit line is calculated between the ED_{84} and ED_{50} values. A high value for slope would represent high value for data certainty, with minimal sample-to-sample variability affecting results.

Results

Positive responses, such as that seen in Figure 3, were recorded for every parameter tested in this project. These viable action potentials obtained from the laser exposures are presented in Table 1. Measurements were taken from multiple subjects and calculated by combining all data for each spot size and wavelength.

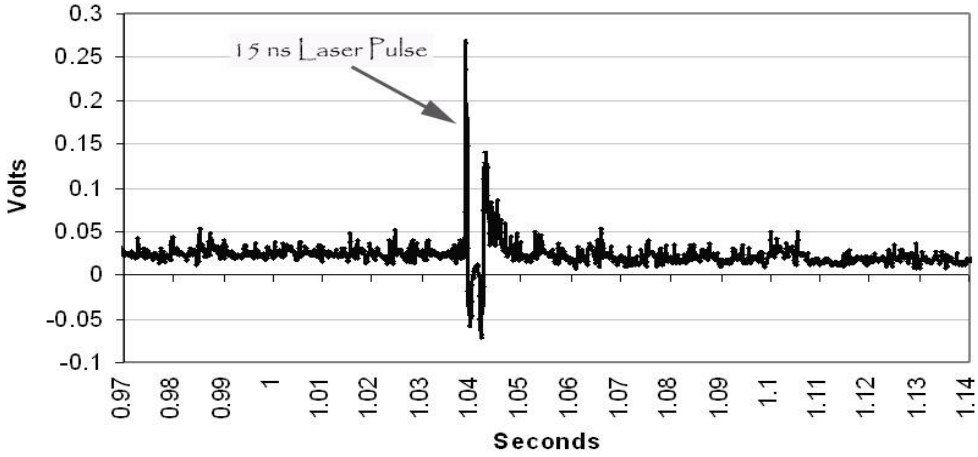

Figure 3: Action potential elicited from a 2-mm exposure on the frog's calf, using the 1540 nm laser at 1.71 J/cm2.

Table 1: Action potential thresholds (J/cm^2). AP_{50} is the energy level at which 50% of exposures will elicit an action potential. UFL = upper fiducial limit. LFL = lower fiducial limit

ACTION POTENTIAL THRESHOLDS								
Spot Size	1064 nm				1540 nm			
	AP50	UFL	LFL	SLOPE	AP50	UFL	LFL	SLOPE
2 mm	0.900	1.262	1.470	5.483	1.331	1.838	1.981	74.783
3 mm	0.497	0.557	0.443	25.910	0.449	0.712	0.195	3.423
4 mm	0.430	0.520	0.297	3.678	0.323	0.350	0.296	37.950

The action potentials elicited demonstrated very similar activation trends among the 1064- and 1540-nm lasers. At each wavelength, the 2-mm spot sizes required approximately 1 J/cm^2 of radiant energy to achieve an action potential versus the larger spot sizes which required less than 0.5 J/cm^2. (Figure 4 and Figure 5) This data also shows that the skin became damaged at levels below AP thresholds for both wavelengths when using a 4-mm spot size. The most notable difference between the two lasers was that skin damage occurred below the action potential threshold when using the 3 mm beam at 1064 nm, but not at 1540 nm. (Figure 4 and Figure 5)

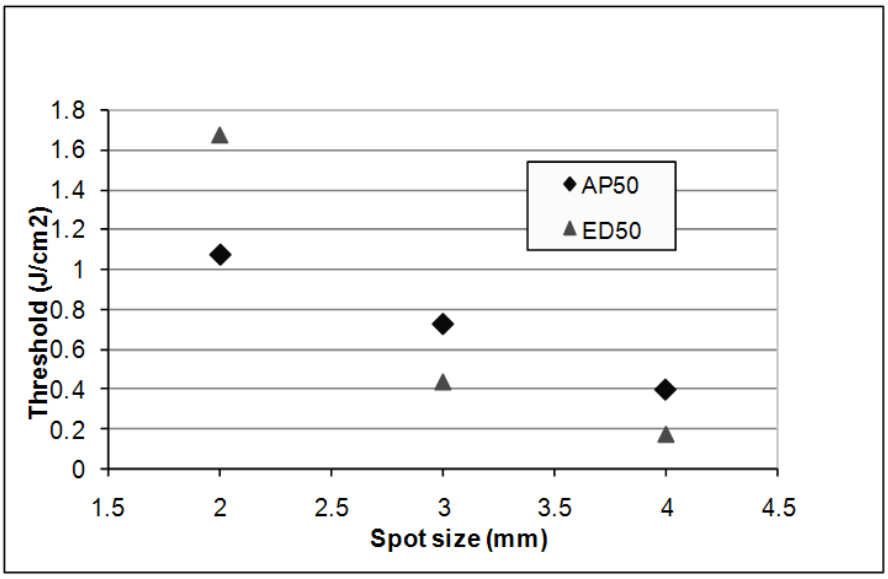

Figure 4: Radiant exposure (J/cm^2) values for skin damage and action potential thresholds at 1064 nm.

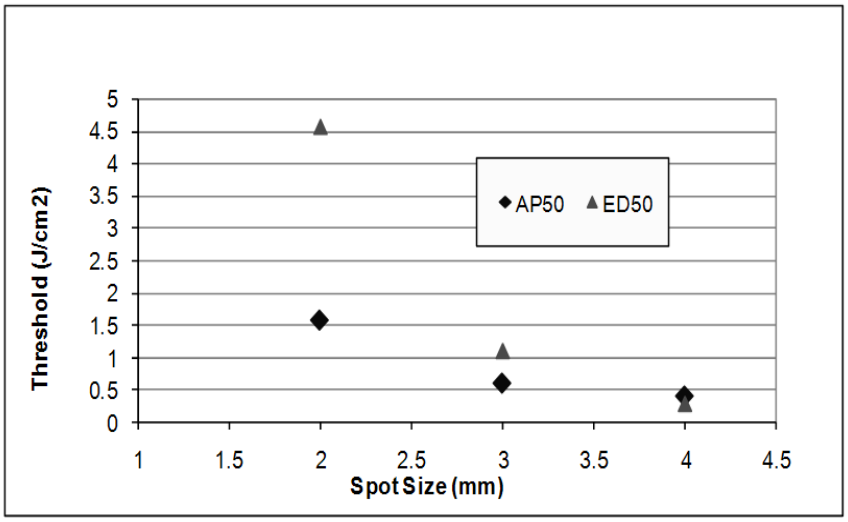

Figure 5: Skin damage and action potential thresholds for radiant energy exposures at 1540 nm.

Discussion

A major impediment to this project was the skin's tendency to ablate, even at very low energy levels (0.169 J/cm^2). As seen in Figure 6a, the ablation was inconsistent and varied depending on pigmentation and location of the exposure site. Dark pigmented tissue required less energy and had larger ablation diameters. Thermal data from these areas show temperature rises as low as 0.689 ^{o}C so ablation due to thermal effects are most likely not the reason for this. Many of these low powered exposures did not exhibit the same charred responses around the crater perimeter as the ablations seen with high radiant exposures. (Figure 6b) The most probable cause, although confirmation of this will require additional experimentation, would be photomechanical damage due to stress confinement.

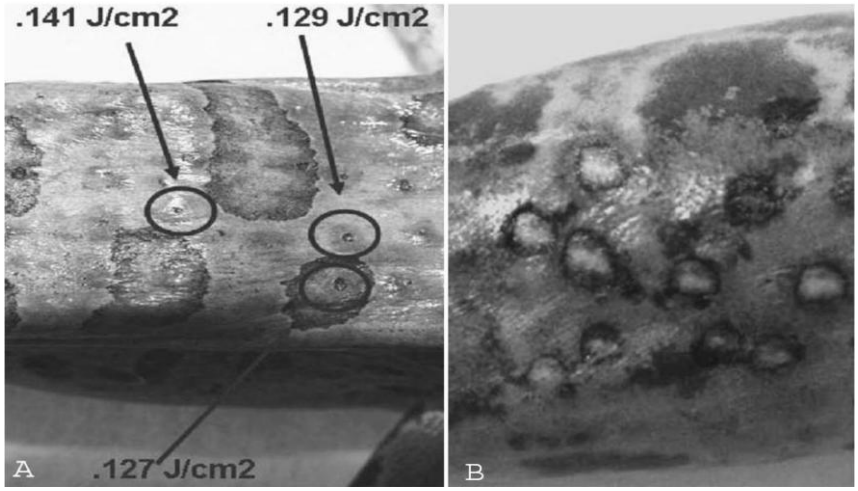

Figure 6: (a) Ablation of tissue on the back of the frog. Similar radiant energy levels (0.127 and 0.129 J/cm^2) show larger amounts of damage for the more heavily pigmented areas than the lightly pigmented areas. (b) Ablation of skin on the dorsal surface of a frog leg. Note the black rings surrounding each crater, potentially indicating charring of the perimeter tissue from thermal effects. Radiant exposure energies varied from 0.386 to 1.12 J/cm^2.

Thermoelastic expansion of tissue by pulsed laser will eject ablated material through stress wave recoil. Stress Waves are produced when optical energy is absorbed into an appropriate medium. If the irradiance is high enough, dielectric breakdown can occur which leads to the formation of high-pressure plasma and the production of large-amplitude stress waves in the tissue. (Dyer & Al-Dhahir, 1990) Shock wave damage effects are due to both compressive and tensile strain. The estimated stress confinement time for this experimental arrangement is 7 μs and 3 μs for 1064 nm and 1540 nm respectively, calculated using Equation 1. (Welch & van Gemert, 1995)

The penetration depth of a Q-switched Nd:YAG laser at 1064-nm is about 1 cm while the penetration depth of a Q-switched Er:glass laser at 1540-nm is around 1 mm (Welch & van Gemert, 1995).

$$\tau_p < \frac{\delta}{\sigma}$$

Equation 1: The criterion for stress confinement is given by where τ_p is the laser pulse length, δ the penetration depth of laser light in tissue, and σ is the speed of sound in tissue.

Since these experiments were conducted using ultrashort lasers, they are certainly within the parameters for stress confinement.

One point to remember is that these animals had very high water content of their skin. Not only did this affect energy absorption, it also influences the amount of heat generated at the exposure site. While we tried to maintain a constant hydration level for the skin, it is possible that some exposures were conducted under drier/wetter conditions than others. To minimize the effects of this, we performed nearly 400 exposures at each spot size. The low temperature changes (< 10 ^{o}C) seen at two of the spot sizes used in this study lend credence to our supposition that ablation

in those areas was due to stress confinement parameters, rather than thermal confinement. (**Error! Reference source not found.**)

Table 2: Spot size versus the average temperature change for each AP_{50} value.

1064 nm	
Spot Size (mm)	Avg. Delta T (C)
2	15 - 20
3	2 - 4
4	10 - 15 0
1540 nm	
Spot Size (mm)	Avg. Delta T (C)
2	6 - 8
3	20 - 40
4	40 - 60

This project was not able to test the actual area stimulated, only the diameter of the laser beam. If the propagation waves were stress induced, it is possible that the wave continued for some unknown distance outside of that diameter to stimulate receptors nearby.

The action potential thresholds achieved at both wavelengths were not very different, despite the distinct penetration depth. This could be due to the thinness of the frog skin; both wavelengths penetrated all the way through the exposed area in many locations. It should be noted again that the thickness of frog skin is much less than that of humans. The average combined thickness of the epidermis, dermis and subcutaneous layers measured in these experiments was 0.367 mm. This value is approximately the thickness of the human epidermis layer alone (Jiang & al., 2002). Therefore, it is difficult to draw direct correlation between results achieved in this study to any expected values for human response. For example, an exposure to human skin using the Er:Glass laser (penetration depth of approximately 1 mm) would just pass the epidermis, but in the case of the frog it penetrated through all layers of the skin. Murine studies already underway should be able to provide a better representation of human skin, allowing for much clearer results.

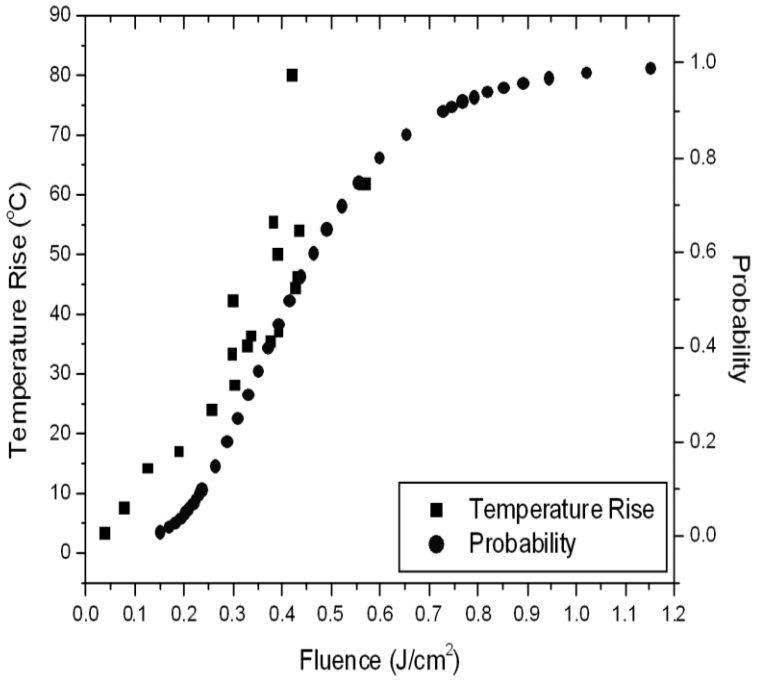

Figure 7: AP_{50} probability vs. temperature rise data for 1540 nm exposures with a 4 mm spot size.

While the parameters of this experiment did not provide any conclusive information, the data from the 4-mm 1540 nm exposures did provide some insight as to future possibilities for this kind of work. This larger spot size provides for a more linear temperature rise, allowing for more predictable energy requirements to estimate surface damage before exposures. (Figure 7)

Conclusions

The results presented in this document are only the beginning of a new line of research in the systematic characterization of neural stimulation with ultrashort-pulsed lasers. For this research we have studied the effects at two wavelengths and three individual beam diameters. The most significant finding provided by this study was that smaller beam diameters were needed to avoid tissue damage while still causing stimulation. As the results show, larger beam diameters have much lower thresholds in terms of radiant exposure for both neural stimulation and skin damage. As the laser beam diameters increased, the damage threshold decreased. The action potential threshold for the larger spots is lower, since the laser is stimulating a greater number of neurons. Therefore, based upon our findings, the ideal spot size would be 3 mm since it required lower laser energy to stimulate action potentials, and did so at energy levels below those that cause skin damage. It was shown that tissue ablation occurred well before the average surface temperature of the skin reached 100° C, which may be explained by laser induced breakdown or stress confinement mechanisms. Indeed, skin damage frequently occurred before action potentials were stimulated at beam diameters of 4 mm for each wavelength. This phenomenon will certainly require additional studies to determine the exact mechanism of damage; whether it be thermal, mechanical, or a combination of the two.

It became obvious that the differences between frog skin pigmentation and morphology from that of humans makes them ill suited as human skin damage threshold models. A mammalian study (currently underway) should provide the necessary data to determine the best wavelength for creating action potentials without causing skin damage. It could also provide more precise data on skin damage, since water content of the skin will not be such an issue.

Finally, this study was conducted with two wavelengths common in the medical and photonics industries. Additional wavelengths should be studied to determine if different penetration depths or powers could yield more optimal results.

Acknowledgement

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